The Bioscan 8(4): 1327-1330, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EVALUATION OF ANTAGONISTIC POTENTIAL OF *TRICHODERMA HARZIANUM* AND *PSEUDOMONAS FLUORESCENS* ISOLATES AGAINST *GLOEOCERCOSPORA SORGHI* CAUSING ZONATE LEAF SPOT OF SORGHUM

JYOTIKA PUROHIT*, YOGENDRA SINGH, SUNAINA BISHT AND A. SRINIVASARAGHVAN
Department of Plant Pathology, College of Agriculture,
G. B. Pant University of Agriculture and Technology, Pantnagar - 263 145, U S Nagar, Uttarakhand, INDIA
e-mail: jyotika.phytopathol@gmail.com

KEYWORDS
Biological control
Gloeocercospora sorghi
Pseudomonas fluorescens
Trichoderma harzianum

Received on :
28.06.2013

Accepted on :
21.10.2013

***Corresponding author**

ABSTRACT
In an attempt to develop effective biocontrol system for management of zonate leaf spot caused by *Gloeocercospora sorghi,* fifteen isolates of *Trichoderma harzianum* and six isolates of *Pseudomonas fluorescens* were tested for their antagonistic potential against *G.sorghi*. Th-43 and Psf-28 isolates achieved maximum inhibition of radial growth of the test pathogen by (77.77%) and (56.66%) respectively under *in vitro* study. Effective isolates of *T. harzianum* (Th-43, 39, 32 and 31) and *P. fluorescens* (Psf-28, 11 and r) were selected on the basis of *in vitro* superiority and further evaluated in glasshouse and field conditions. Treatments were given as three foliar sprays at 35, 45 and 55 days after sowing under *in vivo* conditions (glasshouse and field conditions). In glasshouse conditions, maximum reduction in disease severity was obtained with Th-43 (57%) followed by Th-39 (53.63%) with three foliar sprays. Similarly, Th-39 (36.62%) showed maximum reduction in disease severity with three foliar sprays under field conditions. Thus, the present study demonstrates the possible role of *T. harzianum* and *P. fluorescens* in the induction of antagonistic compounds against *G. sorghi*.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is called as king of millets and is one of the important food and fodder crops in drier parts of India, tropical Africa and China (Rooney and Waniska, 2000). Being cultivated in a range of environments, sorghum is constantly challenged by array of plant pathogens, especially the foliar pathogens. Numerous diseases have been reported in sorghum such as charcoal rot, fusarium root and stalk rot, rough leaf spot, downy mildew, sorghum red stripe and anthracnose (Tarr, 1962). Among them, zonate leaf spot caused by *Gloeocercospora sorghi* is one of the emerging destructive foliar pathogen which causes damage up to 85 per cent of photosynthetic area under humid and cloudy weather conditions (Agnihotri and Pandey, 1977). The characteristic symptoms as roughly circular (or semicircular if they originated near the edge of the leaf) with altering bands of dark purple or red color and tan or straw color, to give a concentric or zonate appearance (Palakshappa and Hiremath, 2003). Foliar infection is commonly observed and generally appears 30 to 40 days after seedling emergence. The estimated yield losses due to foliar diseases in Asia, Africa and America range from 32 to 60 per cent (Sharma, 1980; Frederiksen, 2000). For management of this disease besides many cultural practices chemicals are also used. Chemicals are necessary at present, but are not a long term solution to crop health. Besides their non target effects and hazardous nature, many of them are now losing their effectiveness because of development of resistant strains. Moreover, application of chemicals to sorghum crop is to be avoided as the fodder is fed to the cattle.

Considering the seriousness of problem, the present investigation was carried out to evaluate the efficacy of biocontrol agents (BCAs) *viz., T. harzianum* and *P. fluorescens* against zonate leaf spot of sorghum as an alternative ecofriendly strategy. Among fungi, *Trichoderma* is most researched group and has been found to be useful against this pathogen (Kharayat and Singh, 2012). *Trichoderma* spp. employs various mechanisms like mycoparasitism, antibiosis and competition and has been found useful against aerial and soil pathogens (Weller, 1988; Singh *et al.*, 2011; Bangari *et al.*, 2012; Meena *et al.*, 2012). *Trichoderma* spp. and *Pseudomonas* spp. have been reported to secrete diverse anti-microbial secondary metabolites, which help it in host recognition and pathogen control (Jeyalakshmi *et al.,* 2010; Srivastava *et al.,* 2010; Shanmugaiah *et al.,* 2009; Koche *et al.,* 2013; Singh *et al.*, 2013). With this background, in the present study, attempts have been made to identify antagonistic potential of BCAs against *G. sorghi in vitro* and *in vivo* for the successful management of zonate leaf spot of sorghum to obtain disease free fodder yield.

MATERIALS AND METHODS

Plant material and isolation of fungus

In *kharif* season of 2010, leaf samples were collected from zonate leaf spot infected sorghum plants (Pant Chari-4) from Livestock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. The fungus was isolated on Potato Dextrose Agar (PDA) and incubated at 28 ± 1°C. The growing mycelium from the margin of distinct colonies was sub-cultured on fresh petriplates containing (PDA) to obtain pure culture.

In vitro screening of *Trichoderma harzianum*

Fifteen isolates of *T. harzianum* (Th-1, 2, 4, 6, 9, 12, 13, 14, 19, 22, 31, 32, 37, 39 and 43) were obtained from Biocontrol Laboratory of Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar. These isolates were screened for their antagonistic potential against the *G. sorghi* using dual culture technique (Morton and Stroube, 1955). Five mm disc of four days old culture of *G. sorghi* and test biocontrol agent were kept on to the 90 mm petriplates containing Oat meal agar (OMA) in such a manner that they lie opposite to each other with 6 cm apart. Inoculated petriplates were incubated at 28 ± 1°C for seven days. OMA amended petri plate inoculated centrally with 5 mm disc of test pathogen served as control. Observation on the growth and the ability of *T. harzianum* to colonize the pathogen were recorded after seven days.

In vitro screening of *Pseudomonas fluorescens*

Six isolates of *P. fluorescens* (Psf-4, 6, 11, 18, 28, r) were obtained from Biocontrol Laboratory of Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar and these were screened for their antagonistic potential against the *G. sorghi* following dual culture technique (Morton and Stroube, 1955). Paper discs were cut with the help of punch and sterilized in autoclave at 15 psi for 20 minutes. Five mm disc of four days old culture of the test pathogen was obtained with the help of sterilized cork borer. Sterilized paper discs of 5 mm were dipped in *Pseudomonas* culture then placed in such a manner that both the discs (pathogen and antagonist) lie opposite to each other with 6 cm apart in 90 mm petriplates containing OMA amended with King's medium B (in 50:50 ratio, approx. 20 ml/plate). Inoculated petriplates were incubated at 28 ± 1°C for seven days. OMA amended petriplate was inoculated centrally with 5 mm disc of test pathogen served as control. Observation on the growth and the ability of *P. fluorescens* to colonize the pathogen were recorded after seven days.

Both the *in vitro* experiments were conducted in Completely Randomised Design (CRD) and each treatment was replicated three times. The per cent inhibition of radial growth was calculated using formula given below (Vincent, 1947).

$$\% \text{ inhibition of radial growth} = \frac{\text{Radial growth of fungus in check} - \text{Radial growth of fungus in treatments}}{\text{Radial growth of fungus in check}} \times 100$$

Screening of *T. harzianum* and *P. fluorescens* in glasshouse experiment

Four isolates of *T. harzianum* (Th-43, 39, 32 and 31) and three isolates of *P. fluorescens* (Psf-28, 11 and Psf-r), were selected for glasshouse experiments on the basis of *in vitro* superiority. Three sets of experiments were conducted *viz.;* first set: one foliar spray, second set: two foliar sprays and third set: three foliar sprays. First, second and third foliar sprays were given at 35, 45 and 55 days after sowing (DAS). Ten healthy seeds of sorghum (Pant Chari-4) were sown in pots filled with sterilized soil. Artificial inoculation of *G. sorghi* was done two days prior to treatments. Plants in all three sets were sprayed with *T. harzianum* and *P. fluorescens* isolates @ 10g of spores/litre of water. Observations on disease severity were recorded in 1-9 scale after 60 DAS. Experiments were conducted in completely randomized design (CRD) with three replications.

Field trials

Field experiments were conducted during *Kharif* season of 2010 at Livestock Research Centre, Pantnagar, to evaluate the efficacy of selected BCAs in controlling zonate leaf spot of sorghum. Plants in the field were artificially inoculated two days prior to treatments by spraying the spore suspension of *G. sorghi* containing 5×10^4 spores/ml. The inoculum was sprayed between 6-7 pm as night temperature and humidity were conducive for infection. Four isolates of *T. harzianum viz.,* Th-43, Th-39, Th-32 and Th-31 and three isolates of *P. fluorescence viz.,* Psf-28, Psf-11 and Psf-r were further evaluated in field trial with three treatments *viz.,* one, two and three foliar sprays at 35, 45 and 55 DAS respectively. Field trials were laid out in randomized block design (RBD) with three replications. The observations on disease severity were recorded in 1-9 scale 60 DAS for each treatment taking random samples as proposed by All India Coordinated Sorghum Improvement Project, as follows: (1) Highly resistant (0 to <1% leaf area covered/No symptom); (2) Resistant (up to 5% leaf area covered); (3) Resistant (6-10% leaf area covered); (4) Moderately Resistant (11-20% leaf area covered); (5) Moderately Resistant (21-30% leaf area covered); (6) Susceptible (31-40% leaf area covered); (7) Susceptible (41-50% leaf area covered); (8) Highly Susceptible (51-75% leaf area covered); (9) Highly Susceptible (above75% leaf area covered).

Following formula was used to calculate the per cent disease severity:

$$\% \text{ disease severity (S)} = \frac{\text{Sum of numerical rating}}{\text{Total no. of sample} \times \text{Maximum rating grade}} \times 100$$

RESULTS AND DISCUSSION

In vitro testing of antagonism between BCAs (*Trichoderma harzianum* and *Pseudomonas fluorescens* isolates) and *G. sorghi*

All the 15 isolates of *T. harzianum* reduced the colony growth of *G. sorghi* in dual culture (Table 1). Among them Th-43 performed best which gave 77.77 per cent inhibition of radial growth followed by Th-39 (75.55 %), Th-32 (74.44%) and Th-31 (72.22%), whereas, least inhibition of radial growth was recorded with Th-1 (33.44%). The difference in per cent inhibition of radial growth indicates the difference in their

antagonistic potential for the test pathogen. These observations are in accordance with the findings of Kucuk and Kivanc (2004). *Trichoderma* spp. inhibiting the growth of the pathogen by mechanism of antibiosis has been reported earlier by (Kharayat and Singh, 2012). Similarly, among 6 isolates of *P. fluorescens* evaluated against the pathogen *G. sorghi* (Table 2), in dual culture test, all the isolates reduced the colony growth of pathogen. Among them, Psf-28 performed best which gave 56.66 per cent inhibition of radial growth followed by Psf-11 (53.00%), Psf-r (39.33%), Psf-18 (34.44%), whereas, least inhibition was obtained with Psf-6 (29.66%) and Psf-4 (28.88%). *Trichoderma* spp. and *Pseudomonas* spp. inhibit the growth of pathogens by mechanism of diverse anti-microbial secondary metabolites production (Harman *et al.,* 2004; Bangari *et al.*, 2012; Meena *et al.*, 2012; Singh *et al.*, 2013).

Table 1: Percent inhibition of radial growth of *Gloeocercospora sorghi* by different isolates of *Trichoderma harzianum*

Isolate	Radial growth (cm)	Percent inhibition
Th-43	2.00	77.77
Th-19	2.66	70.44
Th-14	2.56	71.55
Th-32	2.30	74.44
Th-37	2.80	68.88
Th-13	3.00	66.66
Th-39	2.20	75.55
Th-22	3.15	65.00
Th-6	3.33	63.00
Th-1	5.99	33.44
Th-9	3.06	66.00
Th-31	2.50	72.22
Th-12	3.16	64.88
Th-4	4.42	50.88
Th-2	2.82	68.55
Control	9.00	00.00
CD at 5%	0.05	

Table 2: Per cent inhibition of radial growth of *G. sorghi* by different isolates of *Pseudomonas fluorescens*

Isolate	Radial growth (cm)	Per cent inhibition
Psf-28	3.90	56.66
Psf- 6	6.33	29.66
Psf-r	5.46	39.33
Psf-11	4.23	53.00
Psf-18	5.90	34.44
Psf-4	6.40	28.88
Control	9.00	00.00
CD at 5%	0.31	

Glasshouse experiment

Four isolates of *T. harzianum viz.,*Th-43, Th-39, Th-32 and Th-31 and three isolates of *P. fluorescens viz.,* Psf-28, Psf-11 and Psf-r found more effective *in vitro* were further tested in glasshouse condition. In one spray, all the treatments were significantly superior over control. Th-43 was found most effective in reducing disease severity 36.44 per cent followed by Th-39 (33.84%), Th-32 (32.33%) and Th-31 (30.42%). Th-39, Th-32 and Th-31 were at par in reducing disease severity. In case of two foliar sprays, Th-43 (49.30%) was most effective followed by Th-39 (45.76%), Th-32 (45.32%) and Th-31 (44.47%). In three foliar sprays, reduction in disease was maximum with Th-43 (57%) followed by Th-39 (53.63%), Th-32 (52.23%), Th-31(48.13%) and Psf-28 (45.05%) (Table 3). In three sprays, all the treatments were significantly superior over control. *Trichoderma* spp. is the one of the best alternatives for the management of this pathogen due to various mechanisms like mycoparasitism, antibiosis and competition for colonization. Similar to our results, *T. harzianum* has been found effective against a range of economically important aerial and soil borne plant pathogens and is used as biopesticide in green house and field applications (Tondje *et al.,* 2007; Kharayat and Singh, 2012; Srivastava *et al.,* 2010; Bangari

Table 3: Effect of *T. harzianum* and *P. fluorescens* isolates on disease severity of zonate leaf spot with one, two and three foliar spray under glass house conditions

Treatment	Disease severity (%)			Reduction in disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Th-43	35.93	28.66	24.26	36.44	49.30	57.00
Th-32	38.25	30.91	27.00	32.33	45.32	52.23
Th-39	37.40	30.66	26.21	33.84	45.76	53.63
Th-31	39.33	31.39	29.32	30.42	44.47	48.13
Psf-28	41.13	36.06	31.06	27.24	36.21	45.05
Psf-11	42.66	38.40	33.73	24.53	32.07	40.33
Psf-r	44.00	41.06	35.73	22.16	27.26	36.79
Control	56.53	56.53	56.53	00.00	00.00	00.00
CD at 5%	3.45	3.06	1.83			

Table 4: Effect of one, two and three foliar spray of *T. harzianum* and *P. fluorescens* isolates on disease severity of zonate leaf spot under field condition

Treatment	Disease severity (%)			Reduction in disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Th-43	52.02	47.68	40.16	11.22	18.63	31.46
Th-39	49.16	44.51	37.14	16.10	24.04	36.62
Th-32	53.31	47.91	41.71	10.73	18.24	28.82
Th-31	53.41	48.96	43.69	8.85	16.45	25.44
Psf-28	53.18	48.84	43.25	9.24	16.65	26.19
Psf-11	54.00	50.05	43.98	7.84	14.59	24.94
Psf-r	55.02	53.40	46.13	6.10	8.87	21.27
Control	58.60	58.60	58.60	00.00	00.00	00.00
CD at 5%	2.81	3.68	3.71			

and Singh, 2011).

Field experiment

Four isolates of *T. harzianum* and three isolates of *P. fluorescens* found effective in glasshouse were further evaluated in field condition for their efficacy against the pathogen. Foliar spray with *T. harzianum* and *P. fluorescens* reduced disease severity significantly over control. Maximum reduction was obtained with Th-39 (16.10%) followed by Th-43 (11.22%), Th-32 (10.73%) and Psf-28 (9.24%) in case of one spray. In two sprays, Th-39 recorded maximum reduction in disease severity 24.04 per cent followed by Th-43 (18.63%), Th-32 (18.24%) and Psf-28 (16.65%). Three sprays with Th-39 recorded maximum reduction in disease severity by 36.62 per cent, Th-43 (31.46%) was at par followed by Th-32 (28.82%) and Psf-28 (26.19%) (Table 4). Similar findings reported by several workers (Nzojiyobiri *et al.,* 2003; Chen *et al.,* 2005; Tiwari, 2006 and Indira *et al.,* 2006).

REFERENCES

Agnihotri, V. P. and Pandey, S. 1977. Zonate leaf spot of jowar caused by *Gloeocercospora sorghi* and its control through fungitoxicants. *Ind. Phytopath*. **29:** 401-406.

Bangari, G. and Singh, Y. 2011. Evaluation of antagonistic potential of *Trichoderma harzianum* isolates against *Colletotrichum graminicola* causing anthracnose of sorghum. *Pantnagar J. Res.* **9(1):** 70-71.

Bangari, G., Singh, Y. and Singh, V. K. 2012. Assessing biocontrol agents against anthracnose disease and their effect on growth parameter in *Sorghum bicolor* (L.) Moench. *Vegetos.* **25(1)**: 16-20.

Chen, J., Harman, G. E., Comis, A. and Cheng, G. W. 2005. Proteins related to the biocontrol of *Pythium* damping-off in maize with *Trichoderma harzianum* Rifai. *J. Integrative Plant Biol.* **47(8):** 988-997.

Frederiksen, R. A. 2000. Diseases and disease management in sorghum. In: Smith WC, Frederiksen RA (eds) Sorghum: origin, history, technology and production. Wiley, NewYork, pp. 497-533.

Harman, G. E., Petzoldt, R., Comis, A. and Chen, J. 2004. Interactions between *Trichoderma harzianum* strain T 22 and maize inbred line Mo1 7 and effects of this interaction on disease caused by *Pythium ultimum* and *Colletotrichum graminicola*. *Phytopathology*. **94 (2):** 147-153.

Indira, S., Muthusubramanian, V., Tonapi, V. A., Varanavasiappan, S. and Seetharama, N. 2006. Utility of biocontrol agents in the suppression of seed borne pathogenic mycoflora and their effect on seed quality in sorghum [*Sorghum bicolor* (L.) Moench]. *Archives Phytopath. Pl. protec.* **39:** 379-387.

Jeyalakshmi, C., Madhiazhahagan, K. and Rettinassababady, C. 2010. Effect of different methods of application of *Pseudomonas fluorescens* against bacterial leaf blight under direct sown rice. *Journal of Biopesticides*. **3(2):** 487-488.

Kharayat, B. S and Singh, Y. 2012. Biological control of zonate leaf spot of sorghum caused by *Gloeocercospora sorghi*. *Internat. J. Plant Protec.* **5(2):** 401-404.

Koche, M. D., Gade, R. M. and Deshmukh, A. G. 2013. Antifungal activity of secondary metabolites produced by *Pseudomonas fluorescens*. *The Bioscan.* **8(2):** Supplement on Medicinal Plants. 723-726.

Kucuk, C. and Kivanc, M. 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turk. J. Biol*. **28:** 111-115.

Meena, Y. K., Singh, Y. and Kharayat, B. S. 2012. Biological control of anthracnose of sorghum caused by *Colletotrichum graminicola*. *Internat. J. Plant Protec.* **5(2):** 333-338.

Morton, D. J. and Stroube, W. H. 1955. Antagonistic and stimulating effects of soil micro-organisms upon *Sclerotium*. *Phytopathology*. **45:** 417-420.

Nzojiyobiri, J. B., Xu, J., Song, F. M. and Shen, Y. 2003. Resistance induced by *Trichoderma harzianum* NF9 against *Magnaporthe grisea* and *Xanthomonas oryzae* pv. *oryzae* in rice. *Chinese J. Biol. Control.* **19(3):** 111-114.

Palakshappa, M. G. and Hiremath, R. V. 2003. Symptomatology of zonate leaf spot of sorghum caused by *Gloeocercospora sorghi*. *Karnataka J. Agril. Sci.* **16(3):** 408-412.

Rooney, L. W. and Waniska, R. D. 2000. Sorghum food and industrial utilization. In sorghum: Origin, History, Technology, and Production, Eds. C. Wayne Smith and J Tom Cothrew. New York: John Wiley and Sons, INC Publishers. pp. 689-729.

Shanmugiah, V., Balasubramanian, N., Gomathinayagam, S., Manoharan, P. T. and Rajendra, A. 2009. Effect of single application of *Trichoderma viridae* and *Pseudomonas fluorescens* on growth promotion in cotton plants. *Afri. J. Agri. Res.* **4(11):** 1220-1225.

Sharma, H. C. 1980. Screening of sorghum for leaf-disease resistance in India. In: Williams RJ, Frederiksen R. A., Mughogho L. K. (eds) Sorghum diseases: a world review (Proc Int Workshop Sorghum Diseases). ICRISAT, Patancheru. pp. 249–264.

Singh, B. N., Singh, A., Singh, S. P. and Singh, H. B. 2011. *Trichoderma harzianum* mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defense against *Rhizoctonia solani*. *Eur. J. Plant Pathol.* **131:** 121-134.

Singh, S. P., Singh, H. B. and Singh, D. K. 2013. *Trichoderma harzianum* and *Pseudomonas* sp. mediated management of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum* Mill.). *The Bioscan.* **8(3):** 801-804.

Srivastava, R., Khalid, A., Singh, U. S. and Sharma, A. K. 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum f.sp. lycopersici* for the management of tomato wilt. *Biological Control.* **53:** 24-31.

Tarr, S. A. J. 1962. Diseases of sorghum, sudan grass and broom corn. The Commonwealth Mycological Institute, Kew, Surrey, England. pp. 137-141.

Tiwari, I. K. 2006. Studies on zonate leaf spot of sorghum caused by *Gloeocercospora sorghi*. M.Sc. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (INDIA). pp.

Tondje, P. R., Roberts, D. P., Bon, M. C., Widmer, T., Samuels, G. J., Ismaiel, A., Begoude, A. D., Tchana, T., Nyemb-Tshomb, E., Ndoumbe-Nkeng, M., Bateman, R., Fontem, D., Hebbar, K. P. 2007. Isolation and identification of mycoparasitic isolates of *Trichoderma asperellum* with potential for suppression of black pod disease of cacao in Cameron. *Biological Control.* **43:** 202-212.

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* **159:** 350.

Weller, D. M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol*. **26:** 379-407.